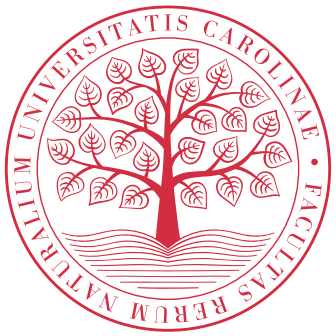

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BACHELOR THESIS

Brain connectivity as a new approach to studying neurodegenerative diseases

Mozková konektivita jako nový nástroj pro studium neurodegenerativních onemocnění

Anna Agafonova

Supervisor of the bachelor thesis: Doc. RNDr. Jiří Novotný, DSc.

Prague 2021

I declare that I carried out this bachelor thesis independently, and only with the cited sources, literature, and other professional sources. It has not been used to obtain another or the same degree.

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Dedicated to all giants whose shoulders I am standing on.

Abstract

Experimental neuroscience was for a long time mainly invasive, focused on the subsystem level: individual cells, cellular populations, proteins or genes. Furthermore, the experiments were mainly done on laboratory animals. Nevertheless, a lot of questions seem to be unanswered with such an approach. One of them is the question of the nature of neurodegeneration. Connectomics suggests a new systematic approach to experimental neuroscience. In this thesis, I have described what is connectomics, what are its methods, and what knowledge is already gained with it; finally, I provided basic information on the issue of neurodegeneration and showed how a connectomics approach can help to answer some questions related to it.

Keywords: Brain connectivity, neurodegeneration, Alzheimer's disease, Parkinson's disease

Experimentální neurovědy byly po dlouhou dobu hlavně invazivní, zaměřené na úroveň subsystému: jednotlivé buňky, buněčné populace, proteiny nebo geny. Pokusy se prováděly hlavně na laboratorních zvířatech. Mnoho otázek se však zdá být s takovým přístupem nezodpovězeno. Jednou z nich je otázka povahy neurodegenerace. Konnektomika navrhuje nový systematický přístup k experimentální neurovědě. V této práci já jsem popsala, co je to konnektomika, jaké jsou její metody a jaké znalosti s ní již byly získány; nakonec jsem uvedla základní informace týkající se problematiky neurodegenerace a ukázala jsem, jak konektomický příistup může pomoci odpovědět na některé otázky s ní související.

Klíčová slova: Mozková konnektivita, neurodegenerace, Alzheimerova choroba, Parkinsonova choroba

Contents

List of Abbreviations

- Aβ - Amyloid beta
- AD - Alzheimer's disease
- AI - Artificial intelligence
- AMPAR - The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
- APP - Amyloid beta precursor protein
- ApoE - Apolipoprotein E
- CFJ - Creutzfeldt–Jakob disease
- CNS - Central nervous system
- CSF - Cerebrospinal fluid
- CST - Corticospinal tract
- CT - Computed tomography
- DMN - Default mode network
- DTI - Diffusion tensor imaging
- EEG - Electroencephalography
- EM - Electron microscopy
- FAD - Familial Alzheimer's disease
- FTD - Frontotemporal dementia
- LOAD - Late-onset Alzheimer's disease
- LTD - Long-term depression
- LTP - Long-term potentiation
- MRI - Magnetic resonance imaging
- NMDAR - The N-methyl-D-aspartate receptor
- PD - Parkinson disease
- SAD - Sporadic Alzheimer's disease

Introduction

There are two pieces of data that inspire this thesis. At first, according to UN 2017 World Population Ageing report, the world population is getting older. As expected, by the year of 2050, the number of older people will double. This significant increase in life expectancy brings new challenges to humanity and one of them is neurodegenerative diseases, which also drastically increase in their occurrence. This means that in the future more people would seek for professional help and reliable remedies. Still, although, the diseases are not understood and considered fatal with a prognosis of 3 to 9 years for Alzheimer's patients.

Second motivation comes from Google Ngram Viewer data collection, according to which the word "connectomics" started to appear near 2004, and then the number of word incidents in the literature increased drastically and there is no doubt it will rise even more. However, although the term is new, connectomics existed for a much longer time, probably since the invention of the first MRI machines. However, only now these and similar machines are reaching the needed speed, quality, and becoming more affordable, and together with them grow our capabilities of fast and reliable analysis of the produced data. Together this is allowing fields like connectomics to finally bloom.

Connectomics experimental approaches have advantages that are crucial for the research of neurodegeneration. It offers noninvasive, in vivo methods that can be done on humans, thus allowing to study patients directly. However, besides of that, it also offers a new point of view on these pathologies.

Some questions cannot be answered with traditional approaches.

- Why is there a cognitive decline in patients with neurodegeneration?
- Why do sometimes symptoms vary between patients?
- Why do different neurodegenerative diseases start in different places?
- How does it spread throughout the whole brain?

For these and other questions, connectomics has answers to offer. And in this thesis, I aim to describe both questions and answers and determine what makes connectomics a nice tool for studying brain pathologies.

1. Connectomics

1.1 Background for connectivity research

1.1.1 Synapses are the basis for neuronal connectivity

Biological sciences have been going through a lot of disagreements among its fellows, but inside neuroscience, one of the most encountered debates dates to the beginning of the last century. Back then, it was claimed that the nervous system represents a single, continuous network, so that it is reticular. Some resisted and claimed that it is contiguous rather than continuous so that there should be connections between separate units. Among the most notable followers from different sides of this conflict were Camillo Golgi and Santiago Ramón y Cajal, who, despite the disagreement, shared the Physiology and Medicine Nobel Prize in 1906 (Jones, 1999). The prize was given for the incredible histological achievements done by Santiago Ramon y Cajal using Golgi's method of staining which showed the presence of connections between two neighboring discrete units, now known as neurons. He presented his great anatomical drawings of many neural system structures and, it served as additional proof to the neuron connectivity hypothesis (Cajal, 1906).

Another great neuroscientist, Sir Charles Sherrington, while working on the textbook of Physiology 1897, suggested the term "synapse" for these connections inspired by its Greek meaning which emphasizes contact to be an active process (Tansey, 1997).

All above became the beginning of the Neuron Doctrine, which certainly dominates in modern science. The Doctrine has established neurons as neural system subunits, much like other cells in an organism but heavily interconnected in between themselves by means of synapses (Jones, 1999).

Neuron Doctrine was further confirmed after investigation of synapse morphology with electron microscopy half a century later. And a synapse is recognized based on the following features:

1. Synapse is found in a place of close contact of two membranes; in this place, membranes are not myelinated as has been revealed on electron microscopy images. Such a place is seen in images as a higher density locality on both membranes. This is most prominent though on the postsynaptic membrane due to the abundant presence of proteins like receptors and supportive machinery. This is referred to as postsynaptic density (Banker et al., 1974). A neuron is said to be polarized and the signal goes in direction from the presynaptic membrane to the postsynaptic one.

2. Rich clusters of mitochondria are also shown to be located in close proximity to the synapse (Palay, 1956), suggesting a higher energy demand in these regions.

3. On a presynaptic membrane in an active zone and close to it dense clusters of synaptic vesicles are located. These vesicles contain neurotransmitters, chemicals that travel through the synaptic cleft, and, thus, transmit signals from one neuron to the other as shown in studies of Bernard Katz and Paul

Fatt. The signal is transmitted in so-called "quants", and release is said to be probabilistic which is consistent with the vesicle functioning (Fatt and Katz, 1952; Katz and Miledi, 1972).

Depending on the neurotransmitter used, synapses can be divided into two categories - excitatory and inhibitory, where the excitatory ones promote postsynaptic membrane depolarization, and inhibitory - hyperpolarization (Eccles et al., 1966, 1954).

Given that two neurons can communicate by means of synapses, the groups of neurons are further organized in chains, or as they are called neural circuits. Donald O. Hebb developed the theory now known under his name. The Hebbian theory associates the functionality of neurons with the structure of their neural circuits (Sejnowski, 1999). He postulated that the electrical activity of neurons is conserved in connections between these neurons by the mechanism of neuronal plasticity, thus, an experience is able to shape the anatomy of the nervous system. With the modern technology of two-photon microscopy, one can now observe these changes in the living mouse brain in real-time and then record synaptic plasticity as an appearance, disappearance, or transformation of dendritic spines, i.e. protrusions of dendrites that typically form synapses with axons (Grutzendler and Gan, 2006). Example of such visualization of synaptic dynamics is shown in Figure 1.

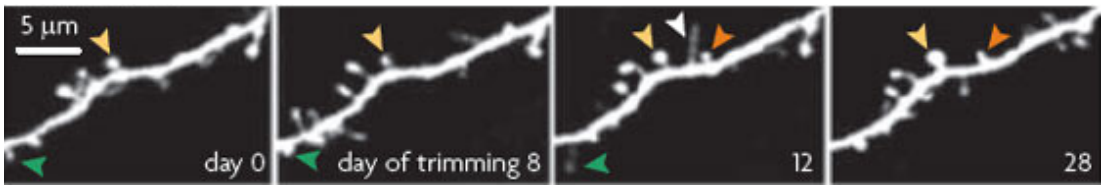

Figure 1: Figure illustrates synaptic dynamics observed with two photon microscopy in mouse barrel cortex neurons after whiskers trimming. Green arrow show the disappearing spines, orange - appearing, yellow - stable. Image is adapted from (Holtmaat and Svoboda, 2009)

The basic mechanism suggested for synaptic plasticity and Hebbian learning is long-term potentiation (Ganong et al., 1986). The phenomenon is described as an increase in the excitability of neurons after repetitive, high-frequency stimulation of these neurons (Bliss and Lømo, 1973). The model for LTP explains how electrical stimulation orchestrate molecular mechanisms that lead to changes seemingly on both pre- and postsynaptic membranes, these mechanisms include AMPAR, that provide initial depolarization, and NMDAR, responsible for an increase of intracellular Ca^{++} levels, and internal second messengers such as CaMKII, that explain long-term changes on a postsynaptic membrane, such as increase in the amount of AMPA receptors, and their conductivity (Bekkers and Stevens, 1990; Collingridge and Bliss, 1987; Bosch et al., 2014). There is also an active mechanism of destruction of existing connections - the mechanism called long-term depression, or LTD. LTD works in a similar manner as LTP operating with the very same molecules but in an opposite fashion, thus, providing possible explanation for mechanisms of forgetting, or disappearance of spines (Collingridge et al., 2010).

Considering all mentioned above, these active connections between neurons everywhere in the nervous system provide a great background for the explanation of many complex phenomena including such as remembering, forgetting, sensing, thinking, but also illnesses of the brain are likely to start with these connections. Connections, or synapses, are organized in chains, and chains that are big enough can be called networks. Such networks are dynamic enough to rearrange under experience but stable enough to maintain the most vital functions. These networks are the subject that connectomics studies.

1.1.2 Methodology of connectomics

In April 2003, the Human Genome Project was completed and thus was resolved one of the greatest challenges of biological science. To fully read the genome means to know all coding sequences of it or genes, and what their functional role is. And in some ways, this definition is similar to the definition of connectomics goals. One of the leading scientists in connectomics, Olaf Sporns in 2005 defined connectome (Sporns et al., 2005) as

> "comprehensive structural description of the network of elements and connections forming the human brain"

In other words, a connectome is a map of all connections or synapses in the brain. This map can be structural if physical synapses exist between two neurons, or functional, if two neurons fire simultaneously, thus serving the same visible cognitive function. And the goal of connectomics is to find this map and learn to read it. Considering the human brain with its up to 10^{15} connections, the second main division emerges. Some, including Olaf Sporns (Sporns et al., 2005), subdivide connectomics as a branch of neuroscience into one that studies the microscale and one that studies the macroscale, and the methodologies of them significantly differ.

The common way of studying structural connectivity on the microscale is the application of electron microscopy. The synaptic cleft is of a size range from 20 nanometres to 40 nanometres and so, it is revealed with a high level of detail with electron microscopy that reaches the resolution much below this value. In connectomics, both transmission electron microscopy and scanning electron microscopy are used. To map all synapses, simple imaging, however, is not sufficient and techniques for visualizing the 3D structure of prepared tissues are used. To do so, cubes of fixed tissue are shot slice by slice. Decades ago, slices were made manually, preserved, and imaged. As the demand for this increased, single automated techniques appeared to substitute manual cutting. First, the microtome was built in to the microscope, allowing to image directly the fresh-cut and reduce manual manipulation of samples. One such established method is serial block-face scanning electron microscopy invented by Winfried Denk at Max Planck Institute (Denk and Horstmann, 2004). Another method uses ionized beams to create slices - the FIB-SEM method (Bosch et al., 2015).

After such a set of images is obtained, nothing is still known about the connectome. To make the reconstruction of local neural circuits, the analysis that will mark the neurons on each slice should be applied. During previous decades, in studies like the one that reconstructed the full connectome of *C. elegans* (White

et al., 1986), this process has to be done mostly manually only with some help of computers; nowadays advances in computer vision helped to automate this process. It is made with a segmentation process, and the selected cell and its axons are used for the 3D reconstruction of the neuron model. Application of machine learning and artificial intelligence further speed up the process. The research group of Sebastian Seung even tries to overcome the problem of big data analysis with crowdsourcing, they have created the public game Eyewire [1] that aims to attract the public to help to control automated analysis, and companies like Google (Li et al., 2019) are participating in connectome decoding by creating new powerful algorithms for segmentation. Since electron microscopy offers such great resolution, it is applied in a big percentage of connectivity research on a microscale. However, this method also has its own limitations, such as that the images obtained are grayscale, and thus the segmentation process is more complicated since it is harder to distinguish the objects of interest. Therefore, some researchers try to overcome this with optical microscopy; with the advantage of fluorescence, they are able to obtain colorful maps that are easier for analysis. Using transgenic strategies, they are able to get many colors from just a few fluorescent proteins by regulating their expression and so combining different patterns of it. Here, Figure 2 shows an example of their results, where each individual neuron possesses its own unique color (Livet et al., 2007).

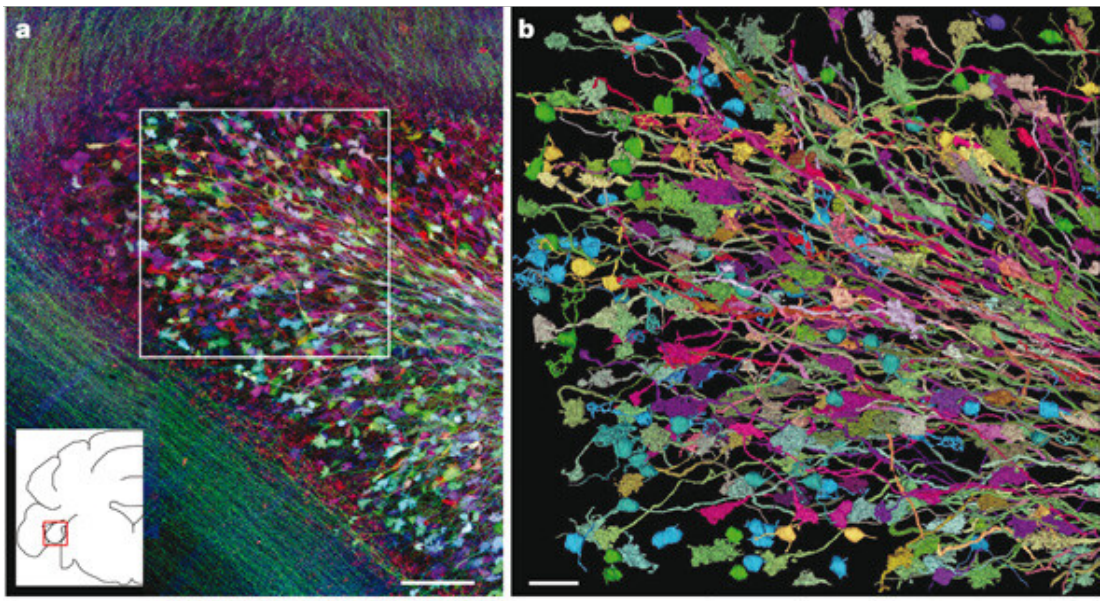

Figure 2: Brain circuits visualized with transgenic staining techniques. From (Livet et al., 2007)

Another method, called array tomography, combines both optical and electron microscopy and visualizes ultrathin slices first colorized by fluorescent stains, and later stained with heavy metals and imaged with electron microscopy (Micheva and Smith, 2007).

The disadvantages of the methods listed above are that they can not be used for revealing structures of bigger sizes, such as even parts of the human brain or mouse brain. The second major disadvantage is that these methods can be only

performed on the dead tissue. And lastly, all techniques involving microscopy are clearly invasive and for more practical purposes, such as research on neurodegeneration, it is a crucial issue.

The most common noninvasive methods that are used in the macroscale are CT, fMRI, PET, and DWI, and its special kind - DTI. These methods allow to visualize the structural and chemical content of the brain.

They appeared to be massively available during the last decades of the last century. X-rays were already a good way to visualize the internal structures of the organisms, however, they were 2-dimensional images and for complex structures like lungs or brain, it provided very little information. However, when multiple such images are combined, the result provides the necessary depth information, thus allowing it to differentiate between fluid and solid structures to a much higher extent. The described method is a computerized tomography or CT technique. Another method, suggested by Paul Lauterbur in 1973, MRI, a technique based on the behavior of protons in the magnetic field, provides an opportunity to differentiate between different types of molecules, as they provide a different environment for protons and, thus, this affects how photons behave in response to radio frequency waves, all in all, enabling to visualize differences in brain structures (Lauterbur, 1973). Both methods are used for describing the structure of the brain on a macroscale; MRI, although, is able to distinguish well between dark and white matter. Considering that white matter consists of myelinated axons, these scans provide some information on connectivity in the brain. Nevertheless, the level of detail is rather very low, and it is impossible to differentiate between individual axonal pathways or even some bundles in the white matter. This can be also observed in Figure 3 which provides an example of an MRI scan.

To overcome this, the diffusion tractography or DTI method was invented; this method is often applied for visualizing long-range structural connectivity. In this method, the properties of MRI are used to measure the degree of anisotropic diffusion, as this type of diffusion occurs along elongated pathways, it reveals the localization and directionality of individual axon bundles (Conturo et al., 1999). Figure 4 illustrate this method capability with the visualization of corpus callosum tracts. This method is an improved version of DWI, which is only able to measure if there is a constraint for diffusion in tissue. Some more novel methods like Diffusion Spectrum Imaging, DSI, or High angular resolution diffusion imaging, HARDI, aim to further improve these techniques for axonal pathways visualization (Tuch et al., 2002).

For the description of functional connectivity, fMRI, PET, or EEG are used. These techniques are called functional brain visualization because they allow us to show not how the brain tissue is organized but what areas are active in a single moment. To perform this, fMRI utilizes a phenomenon called BOLD or Blood Oxygen Level-Dependent effect discovered by Seiji Ogawa (Ogawa et al., 1990). This phenomenon means that active neurons use more oxygen than nonactive, and, thus, investigators can visualize the magnetic behavior of oxygen-carrying hemoglobin and oxygen-free molecules. PET technique as an alternative to fMRI also provides functional information about the brain. PET can be used with a great variety of different biomarkers, the most popular is the radioactive version of glucose, fludeoxyglucose, or FDG for short. As glucose travels primarily to the

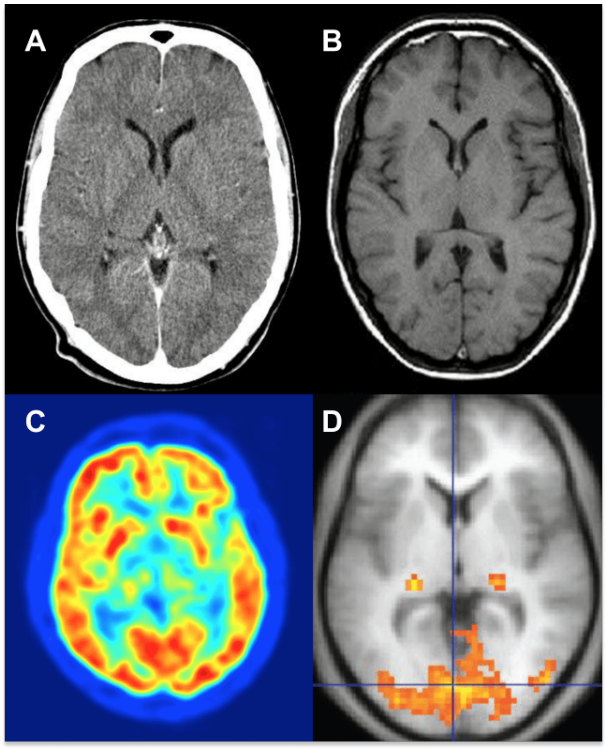

Figure 3: A) CT scan, B) MRI scan, C) PET scan, D) fMRI scan. Adapted from Wikipedia [5].

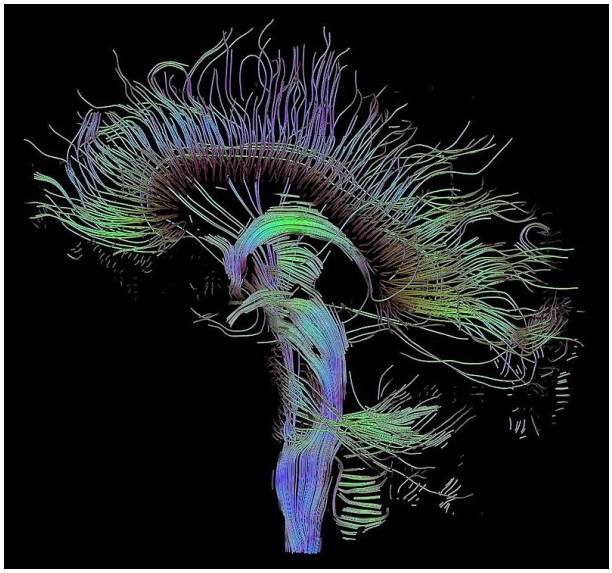

Figure 4: Example, of DTI image of corpus callosum that connect both brain hemispheres, and brain stem. Adapted from Wikipedia [6].

brain regions which are more active, this method also can indirectly show which regions and to what extent react to different kinds of tasks. By experimenting with different human activities, these tools reveal what brain regions are organized in functional units and show a response for the same activity.

Figure 3 shows the comparison between different types of classical brain imaging techniques.

All together, modern techniques provide a large variety of tools for visualizing brain connectivity. However, to gain a full connectome information, one should be able to analyze and integrate all obtained knowledge. This can be done with network science approaches.

1.1.3 Network neuroscience

Here, connectomics intersects with network science, the subject of which greatly varies, since networks are all social connections, protein interaction maps, the internet, or the brain. The main tool that network science uses is the mathematical field of graph theory. It can be said that graph theory was first mentioned by Leonhard Euler in the 18th century when he introduced the problem of Seven Bridges of Konigsberg (Hopkins and Wilson, 2018). As the name suggest, graph theory explores the mathematical structure that is called a graph, where the graph is an ordered pair of vertices and edges or, in mathematical notation

$$G = (V, E)$$

where V is the set of all vertices
$E \subseteq \{x, y | x, y \in V, x \neq y\}$ is a set of edges

In this sense, the neuronal system is a perfect graph as it consists of vertices or neurons, and edges or synapses. A graph can further be undirected or directed, if the connection exists from node V_1 to V_2 and back, or only in one direction, respectively. There are a series of terms central to graph theory, and further, we will review some of them.

One of the most useful ways to describe a vertex is to count how many edges, thus other vertices, connect to it, this number is called the *degree of a vertex*, and it carries the information about the role that each particular vertex plays in the whole network. One can look at all vertices and calculate the degrees for each, then create a degree distribution for the graph. This simple measure will already tell a lot about the graph as will be seen later on in the example of hubs and rich clubs.

In graph theory, one of the most popular problems is the problem of cost optimization. Suppose that each edge costs a particular price, and one has an n number of vertices to connect, the problem puts a question:

> "What would be the optimal way to connect vertices, given that we want to have the lowest cost possible?"

In biological systems, it seems to be closely connected to a path length, or a distance between two neuron bodies. As axons and dendrites fill in the physical space inside the body, and their maintenance takes some resources from this as

well, they put a certain constraint on the nervous system during its development and evolution. This can be also seen from the perspective of wiring cost and graph theory. And, indeed, it was shown in *C.elegans* and, even in larger brains, in part of the brain called lamina cartridge of *Drosophila melanogaster* that the neuron's layout is partially guided by cost optimization as different models of cost optimization can predict the real layout of neurons (Chen et al., 2006; Rivera-Alba et al., 2011).

To describe a graph, instead of drawing it, which would be too complex for bigger graphs, connectivity (adjacency) matrices are used. Suppose a matrix A of the size $n \times n$. In such a matrix, the indices would be assigned to each neuron, and the value $a_{ij} \in A$ would show the number of connections between neurons with indices i and j. Or a matrix can be binarized, so that a matrix would consist of only 0 and 1, so to assign 0 for no connection in between two neurons, and 1 if at least 1 connection exist.

Most of the real-world graphs are said to be complex networks as opposed to random graphs or regular lattices (Bullmore and Sporns, 2009). Complex graphs show up certain properties, which can be described and analyzed. Examples of complex graphs are scale-free and small-world graphs. A small-world graph was introduced by Watts and Strogatz in a 1998 paper (Watts and Strogatz, 1998) as a graph whose topology is somewhere in between a regular graph and a random graph. Figure 5 shows the illustration of such graph. This graph is characterized as having a high clustering coefficient, where the clustering coefficient is the measure of graph nodes tendency to cluster, or to be fully connected inside a given neighbourhood. The term small-worldness refers to the commonly known phenomenon of six handshakes, or, in other words, to the fact that each node can be reached from another node by relatively few steps through their intermediates. This relative closeness is the main feature of small-world graph. Such graphs, they argue, are met everywhere in the real world including *C. elegans* nervous system.

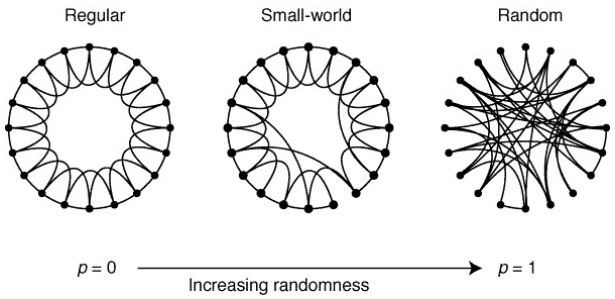

Figure 5: Illustration of small-world graph. Adapted from (Watts and Strogatz, 1998)

Other two important concepts in the field of network science are *hubs* and *rich clubs*. The already mentioned degree distribution for a particular graph can show up if the graph is complex or random. For a random graph this distribution would be uniform, but for nonrandom complex graphs the distribution can be Gaussian or power-law. This is due to the existence of nodes with a high degree in the network, in other words, of hubs. The rich club appears when such hubs are more interconnected in between themselves than with other nodes. Since the creation of rich clubs accounts for a high wiring cost, some speculate that

it can be seen as a trade-off between network constraints and biological function (Towlson et al., 2013). Studies show that neurons in rich clubs are often displaying functional centrality comprising information from many inputs; they are able to evaluate it and give a single output, as a central motor neurons in *C. elegans* (Towlson et al., 2013), or superior frontal cortex in humans (Heuvel and Sporns, 2011).

The similar definition is the graph module. In a graph, a module is a group of nodes with a maximum number of edges within the group and a minimum number of edges with other groups outside (Rubinov and Sporns, 2010). Optimization of a network according to its wiring cost by itself entails reorganization of a network into modules. But as we will see it may be also explained from the organism's biology and anatomy constraints, so as to be shaped by functional needs (Pan et al., 2010).

1.2 Caenorhabditis elegans connectome and microscale connectivity

Still up to today, *C. elegans* is considered to be the only animal for whom we have a full complete connectomic map. This work started under the name of Sydney Brenner, who popularized *C.elegans* as a model organism. Back then, the map was created with an enormous effort by his team who mapped each neuron and all connections almost manually through a huge dataset of EM micrographs (White et al., 1986). Though the map that they have made was still incomplete and the work was fully done after additional efforts of other scientific groups (Chen et al., 2006). Firstly made for hermaphrodite, now it is available for both sexes, male and hermaphrodite (Cook et al., 2019). This work resulted in a full *C.elegans* connectome dataset which is publicly available [2] today. The result mapped all 302 neurons, 132 muscles, and 26 non-muscle end organs with its 4,887 chemical synapses, 1,447 electrical junctions, and 1,410 neuromuscular junctions for hermaphrodite, a slighter bigger number of nodes is observed for male species and, as authors mention, data for both sexes are similar but not identical. The biggest differences are in mating behavior-related neuronal pathways (Cook et al., 2019). The knowledge of the precise positions of each neuron has given us the ability to analyze the data with graph theory and to look for the functional and evolutionary meaning of the observed structure.

The full map, even for as little as 300 neurons, is difficult to comprehend and requires deeper analysis and certain commentary. The nervous system is organized to provide a hierarchical information flow from sensory neurons (here and further in Figure 6 shown as triangles of different colors) through interneurons (hexagons) to the output motor neurons (oval or circles in Figure 6) and then to muscle or nonmuscle end organs (shown as rectangles). Sensory neurons are described based on the stimuli they react to. Interestingly, from the degree distribution of sensory neurons, we do know that it has a wide set of high degree neurons, and it is estimated that information from sensory neurons is able to reach around 70-98 % of all neurons. The interneurons are distinguished into 5 classes based on the output and pathways that they facilitate. Motor neurons make almost half of the whole nervous system, most are connected to somatic

muscles through neuromuscular junctions, and they can be placed in 46 different classes (Cook et al., 2019).

The nervous system of *C.elegans* is a complex network, because of the small average path length and it has high clustering coefficients when compared to a random graph (Varshney et al., 2011). To generalize, similar findings were also gained for rat cortical neurons and for human brain connectome (Hagmann et al., 2007; Song et al., 2005). The knowledge of that allows us to compare *C.elegans* nervous system with other complex networks that occur in real life. Also, for complex networks, it is known that nodes are organized into clusters, modules, and rich clubs. Studies that examine the modular composition of *C.elegans* nervous system show that it exhibits a strong modular topology and that these modules are originating from functional rather than anatomical constraints (Sohn et al., 2011; Pan et al., 2010). This is shown mainly because the neurons in specific clusters do not overlap with neurons within specific ganglia, but it is more likely in one module one will find neurons participating in one functional role.

Let us take a closer look at a *C. elegans* example, ALM, AVM, PLM, and PVD - specific sensory neurons (triangles in the Figure 6), functionally provide sensing of touch, and they fall into a single cluster; this cluster in its turn, contains also command interneurons, AVD and PVC (as some of the hexagons in the same Figure 6), which transmit mechanosensory inputs to motor neurons (Sohn et al., 2011). The similar thing is shown for other functional roles as well. Sohn also shows how these functional modules are organized on a higher level (Sohn et al., 2011), so how separate modules are further integrated to create more complex behaviors. And the study of the rich club organization of *C.elegans* nervous system shows that interaction between modules is facilitated by rich clubs and helps to integrate functional subunits to organize one consistent response, as in the example of organized locomotion (Towlson et al., 2013).Thus, it shows that examining modular composition and clustering can help us understand better how the behavior is born.

The aim of further research would be to translate rich structural information into an understanding of *C.elegans* behavioral patterns like feeding, mating, locomotion. One study, for example, showed which neurons and in what way interact to produce klinotaxis (Izquierdo and Beer, 2013). This was done by search through paths from chemosensing neurons to neck motor neuron, and authors have shown how sensing neurons are able to estimate a gradient fall, transfer the information to interneurons, which synchronize with the oscillatory movements information, and affect this movement according to the following information about chemical gradient. Similar work was also made for mating behavior in *C. elegans* (Jarrell et al., 2012). Another study used network control principles to predict, which particular neurons in a network are crucial for locomotion, and, in general, how many classes of neurons are required for successful locomotion (Yan et al., 2017); notable, they were able to control this prediction using experimental methods, thus, proving that network science predictions are a valuable source of information about the nervous system.

All in all, there is plenty of what we can learn from knowing *C. elegans* connectome. First of all, the connectome of smaller animals gives an opportunity to check the methodology. Thus, successes here should motivate to use the same approach further. Secondly, even though the *C. elegans* connectome was ob-

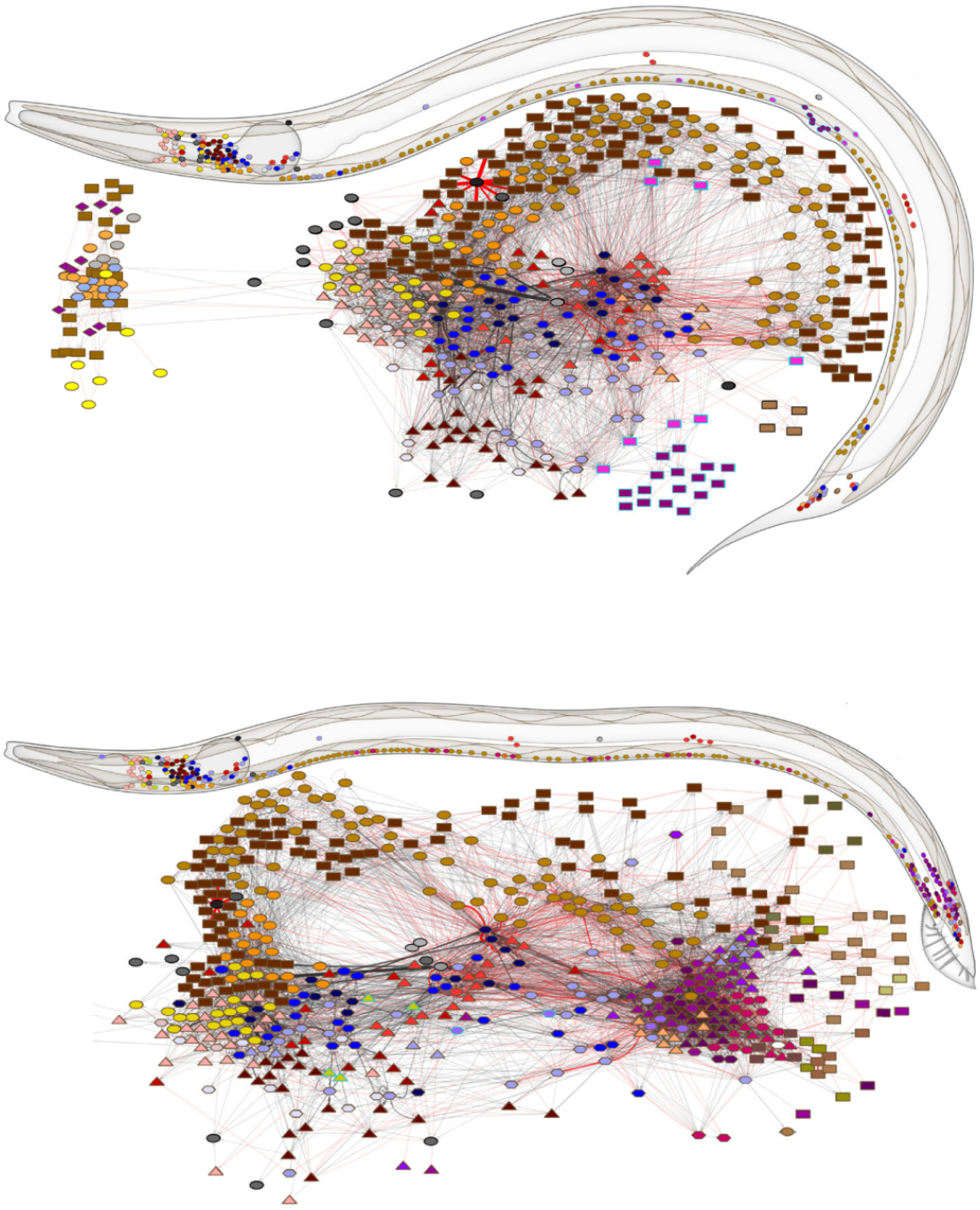

Figure 6: Map of connections of C. elegans nervous system. Above shown hermaphrodite species, and below is male. Adapted from (Cook et al., 2019)

tained from electron micrograph reconstruction, which seems nearly impossible for now to perform on the human brain, a lot can be understood about the human brain from this work. Authors also point out that some properties like degree distribution or some motif frequency show similarities with other studies of the mammalian cortex (Chen et al., 2006).

1.3 Human connectome

Before going to macroscopic structural and functional brain organization, it is worth mentioning that some are also studying mesoscopic connectivity. Such studies reveal the finest details of local circuits and details of microarchitecture in larger brains. Like, for example, the study of fine columnar architecture in rats reveals that excitatory neurons inside one column of the cortex have their own connectivity patterns with selective connections to specific layers (Loftus and Anderson, 2005).

Another study used DTI to show the precise structure of thalamocortical connections in humans (Behrens et al., 2003). This helped not only to define strictly the thalamus-cortex connectivity but also shed light on thalamic cytoarchitecture where it is easier to use projections to the cortex to define functionally distinct nuclei or subregions of thalamus than to use any other non-invasive method. Figure 7 shows the thalamic division based on this method.

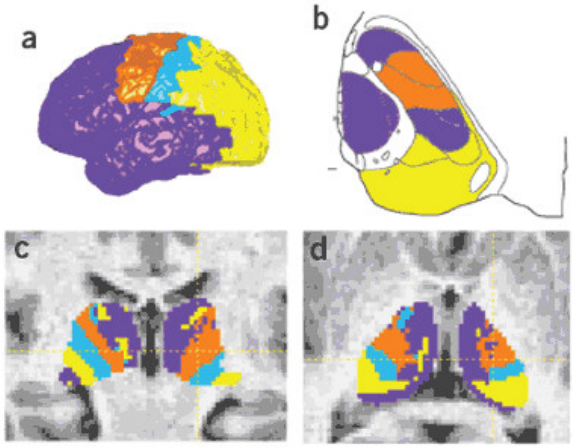

Figure 7: The resulted segmentation from (Behrens et al., 2003), (a) cerebral cortex divided by anatomical landmarks, (b) axial section of thalamus based on histological atlas, (c) and (d) show predicted by connectivity division of thalamus. Color code define connected part to the respective area in cortex.

Similar works are done on different levels of detail and for different areas of the brain. In the future, such studies could be integrated into studies of large-scale connectivity and explain what internal architecture makes it possible for these units to act as a whole and carry out their functional role. As well as it would improve the precision and overall quality of larger-scale works.

The goal of macroscopical structural connectomics is for the whole human brain to define its regions with precise border discrimination and the connections between these regions. A similar goal was already achieved for the primate's

cortex (Felleman and Essen, 1991) or cat's cortico-thalamic system (Scannell et al., 1999). However, due to its enormous size, most of the macroscopical structural studies still work only on a part of it. Mostly, only some parts of white matter are described. The main white matter tracts extracted with DTI are the cingulum, fornix, corpus callosum, the corticospinal tract (CST), the uncinate and superior longitudinal fasciculus, and the inferior and fronto-occipital fasciculi.

To collect and integrate all data into one database that could be further used for analysis, the Human Connectome Project [3] was initiated in July 2009, it was planned to be completed in 2018, however, is still in progress.

Anatomical data alone, however, are insufficient to fully reveal brain organization principles. A lot of useful information comes from fMRI or EEG studies. These studies show which areas of the brain "communicate" with each other during each specific task, even though not always anatomically directly connected. Resting-state fMRI reveals general properties of functional networks as opposed to studies of task-dependent activity.

One of the most studied functional networks is the default mode network (DMN). It consists of areas that are suspended during task-related activities (Raichle et al., 2001). These regions include the anterior and posterior cingulate, the lateral parietal lobes, and the medial and lateral temporal regions, Figure 8 shows the fMRI resting-state scan showing DMN regions. Some of these areas include the highly connected structural and functional core of the brain - the posterior cingulate cortex and precuneus (Utevsky et al., 2014; Hagmann et al., 2008). They are shown to be involved in the retrieval of autobiographic memories and maintenance of the arousal state. The whole network is speculated to be involved in the formation of self, social abilities, abilities to reflect about the past and to plan (Andrews-Hanna, 2012).

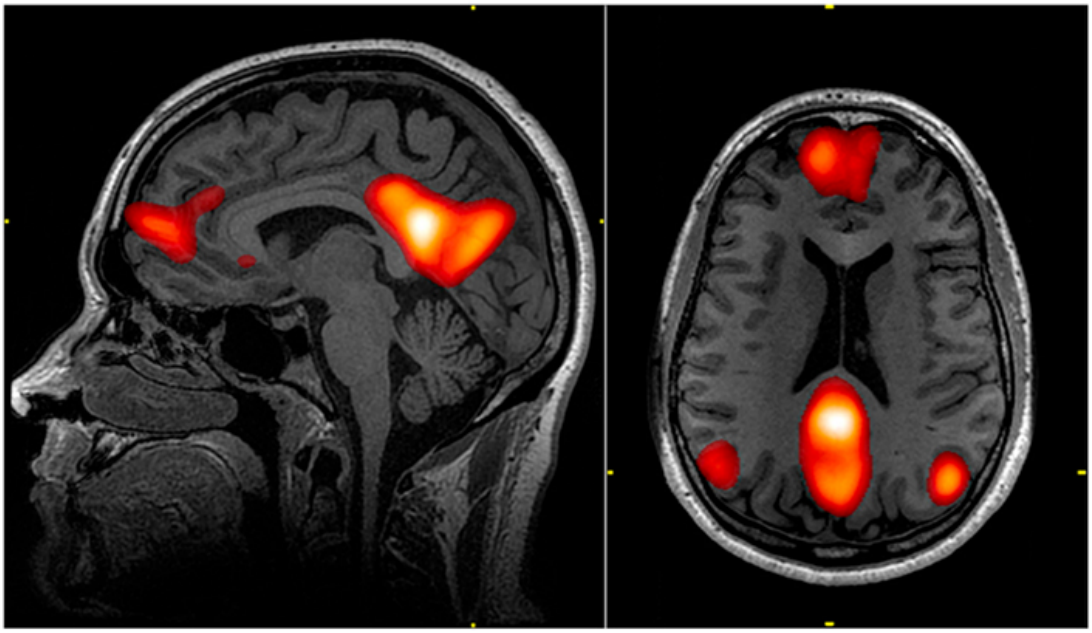

Figure 8: fMRI scan of DMN regions; the medial prefrontal cortex, the posterior cingulate cortex/precuneus, and the angular gyrus. Adapted from Wikipedia [7].

Other important functional brain networks include, for example, the salience network. Its main components are the anterior insula and dorsal anterior cingulate cortex. The network is called "salience" as it is claimed to perceive what stimuli are salient or meaningful to the brain, so then it can organize resources around the detected important stimuli. It is involved in switching between DMN and other networks, so it aims to regulate behavior states (Jilka et al., 2014). There are many other different resting-state functional networks that are currently known. Some of them are shown in the Figure 9 and include language network, sensoromotor network, and visual networks.

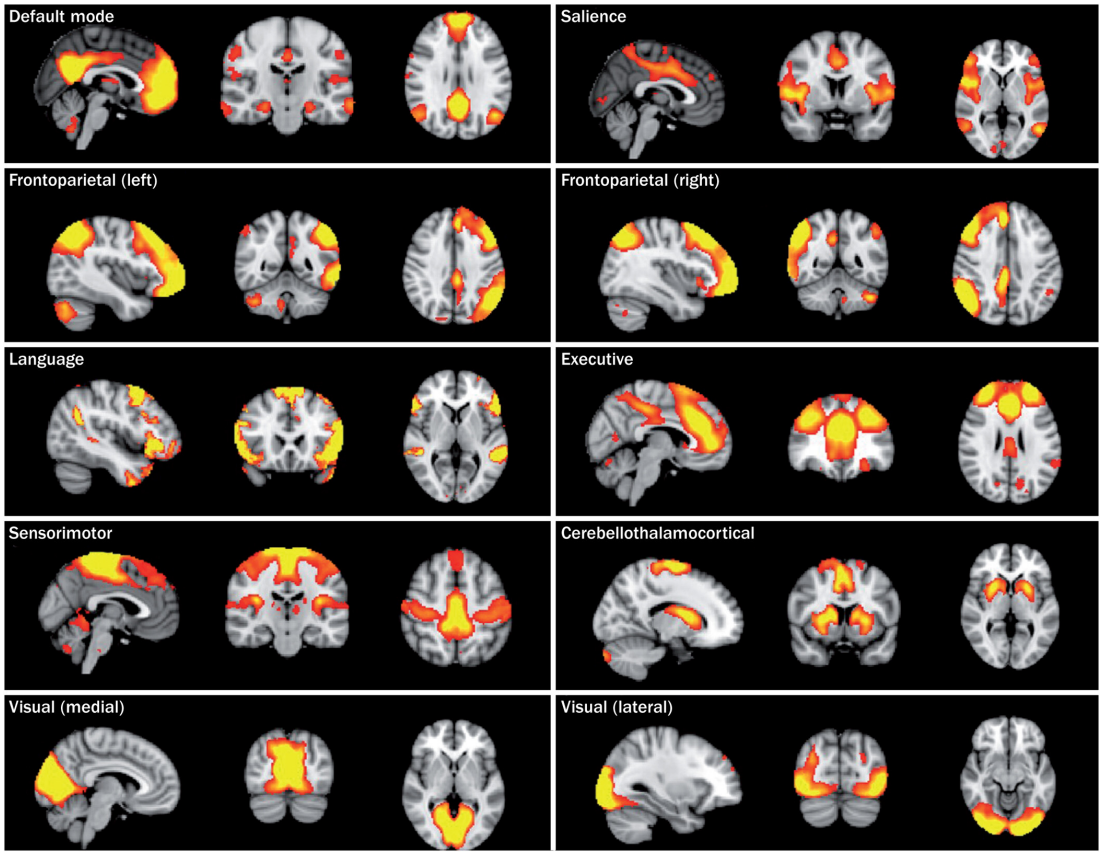

Figure 9: Functional resting-state networks of human brain. Adapted from (Pievani et al., 2014)

Besides structural and functional connectivity, Karl Friston (Friston, 1994) suggested the measure of effective connectivity. As he points out, the functional connectivity is a simple measure of correlation between two or more activities of units. And the correlation says nothing about the actual relationships between them. Effective connectivity aims to provide missing information about how one subunit affects another.

To sum up, a lot of data for human connectome were obtained. Now all of them require to be better integrated together, so the analysis of it would reveal further insights on human nervous system functioning, and in particular, on human cognition. Some simple analysis already revealed that human brain exhibits small-worldness properties (Hagmann et al., 2008) with exponential degree distribution. It is also shown to have hubs and rich clubs, and, as authors speculate,

the disturbances to these central hubs could potentially lead to neuropathologies (Heuvel and Sporns, 2011).

2. Neurodegeneration

2.1 Overview

Neurodegenerative disorders have been studied for many decades. And for all this time, the most known symptom of neurodegeneration was ongoing dementia, which is a sharp cognitive decline over years. The earliest descriptions of age-related dementia are known from all ancient cultures. And at the end of the 19th century, it became an interest for doctors and neurologists who focused on the postmortem examination of the brain tissues from patients with such dementia.

One of the most known cases is the case of Auguste D. which was described in the paper in 1907 by a German professor Alois Alzheimer and the disease was subsequently named after him (Strassnig and Ganguli, 2005). He worked with her during all 4 years of disease progression and examined her brain after her death and found prominent fibrillar tangled structures inside of otherwise normal cells. He also described general changes of the brain, such as cortex thinning due to loss of cells. Up until now, it is known as the main sign of Alzheimer's disease (AD) neurodegeneration.

Besides AD, there are several different pathologies considered neurodegenerative disorders. They are all quite distinct from each other and each occupies its own brain area; thus, for example, frontotemporal dementia, or FTD is very similar to Alzheimer's in molecular signs but occurs in the frontal brain area while AD is prevailing in a temporoparietal lobe. Figure 10 shows the localization of different neurodegenerative pathologies in a brain. Modern medicine shows also that they differ in their symptoms as well. While FTD, for example, affects widely speaking ability and behavior, AD - cognitive abilities, and Parkinson's disease (PD) and Huntington's disease - motor functions.

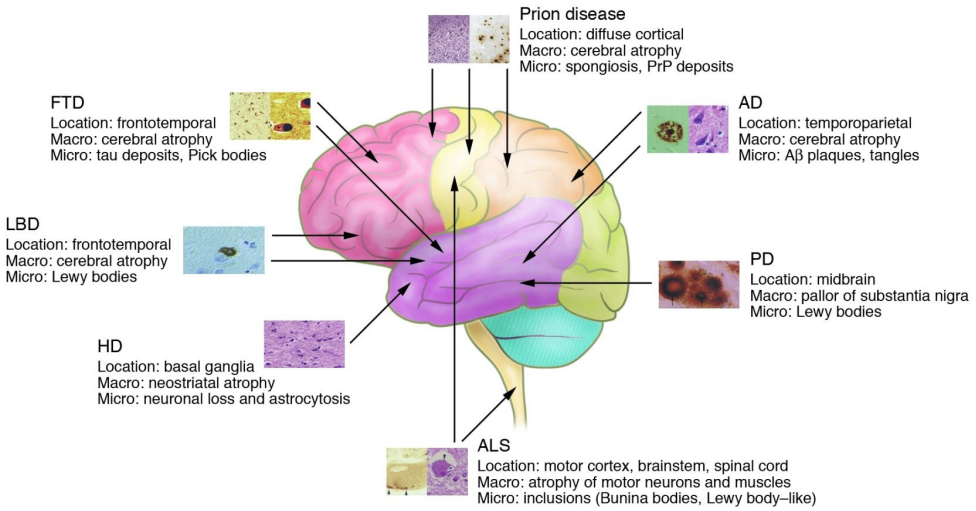

Figure 10: Localization of the main source of pathology for different neurodegenerative disorders. Adapted from (Bertram and Tanzi, 2005)

During the century after this first description of AD pathology, a lot more

was learned about this and other neurodegenerative disorders. Although they are different in many ways, some facts remain the same and allow us to study the core principles of neurodegeneration. Many important breakthroughs were made due to technological progress, which enabled researchers to conduct experiments before considered impossible. First of such, signs of progress were made in histological and staining techniques, like the development of immunohistochemistry, for example, allowed to better characterize the aggregation of misfolded proteins.

The development of visualization techniques, such as MRI and CT, gave scientists the ability to look into the living brain, the invention of better microscopes led us to see these pathologies in dead tissues much closer, and progress in genetics opened a new way to look at the causes of these pathologies (Young, 2009).

The main processes leading to neurodegeneration are malfunctioning of genes, protein misfolding, and network disruption; and they are to be described in the following subchapters.

2.1.1 About misfolded proteins and their aggregation

Since the Alzheimer's observation, it is clear that neurodegeneration and subsequent symptoms correlate with pathogenic protein deposits. This observation was confirmed by many other works as well, including the description of the Prague group that was led by Oscar Fisher and Arnold Pick (Boller and Forbes, 1998). Similar deposits were observed also in pathologies that differ from AD, like Parkinson's disease and dementia with Lewy bodies, described by Frederic Lewy (Engelhardt and Gomes, 2017).

In case of AD pathology, these deposits could be extracellular or intracellular. Extracellular located deposits are called amyloid plaques. They are composed of misfolded amyloid beta (Aβ) protein (Allsop et al., 1983; Glenner and Wong, 1984). This protein that originally functions as a part of transmembrane amyloid beta precursor protein to provide maintenance and growth for neurons but after cleaved it forms fibrils that are components of amyloid plaques (Lu et al., 2013). Both amyloid beta precursor protein and amyloid fibrils are illustrated in the Figure 11. Once manifested plaques show local toxicity on the nearby axons and attract microglia, brain immune cells, to the site, causing subsequent inflammation (Meyer-Luehmann et al., 2008). These plaques also demonstrate a great variability in terms of their structure (Tycko, 2015). This variability can be the explanation of the clinical signs heterogeneity. Even though there is a high correlation between plaques and AD it is important to note that some symptoms-free patients also exhibit mild amounts of amyloid plaques (Iacono et al., 2008), suggesting that it is not a key step in the neurodegeneration process.

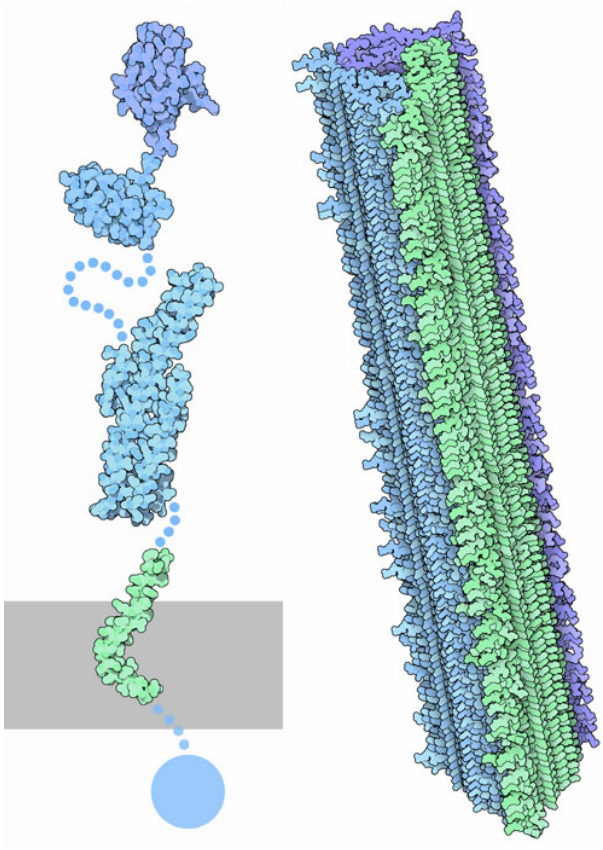

Figure 11: Illustration of amyloid beta precursor protein (on the left) and amyloid beta fibril from a patient with Alzheimer's disease (on the right). Images from Protein Data Bank [4]

Intracellular deposits are neurofibrillary tangles, composed of tau protein (Goedert et al., 1988). Tau proteins that usually serve to stabilize microtubules essential for neuron functioning during the pathology progression are found to be phosphorylated and lose their ability to bind microtubules (Bramblett et al., 1993). Neurofibrillary tangles show higher correlation with cognitive decline in patients (Bennett et al., 2004) which is consistent with the fact that amyloid plaques appear as a first step of neurodegeneration, and with the mentioned fact that plaques are observed in patients with no signs of ongoing dementia.

Not fully clear, however, stays the origin of initial protein misfolding and aggregation. There is a piece of evidence, for example, for the genetic component of amyloid plaque formation (discussed in the next chapter), this is, nevertheless, not comprehensive. When this process is taking place too much, the brain does not manage to mitigate and the neuron is affected by its surroundings. It seems that when it does happen, the mentioned phosphorylation of tau occurs and neurons become disrupted from inside as well, what potentially could trigger cell death. Thus, initial fibril formation could be the first step towards a sequence of events leading to cell loss and network disruption.

The similar was shown for other neurodegenerative disorders as well. Besides amyloidopathies, synucleopathies occur. They are characterized by aggregation of α-synuclein into fibrils and subsequent formation of Lewy bodies.

2.1.2 Genetics of neurodegeneration

It was initially suspected that AD can be caused genetically because of quite similar disorders noticed in people with Down syndrome (Masters et al., 1985). By the time of first genetic research, it was already a fact that Down syndrome is caused by a trisomy of the 21st chromosome, thus, the 21st chromosome was the main suspect for carrying the mutation that causes AD. And soon it was localized to be a gene of APP the cleaved product of which is the main constituent of amyloid plaques (George-Hyslop et al., 1987; Goate, 1991). Together with this gene PSEN1 and PSEN2 were identified (Sherrington et al., 1995). Presenilins are proteins that form a γ-secretase complex. This secretase is responsible for the cleavage of an APP and release of $A\beta$.

These studies, unfortunately, targeted only the familial form of Alzheimer's disease. This is a rare type of AD with a strong rate of inheritance and typically an earlier onset. For the sporadic form of AD, 21st chromosomes usually carry a normal version of the APP gene, such a sporadic form is also referred as Late-Onset Alzheimer's disease (LOAD). For this late-onset type a long time, only one genetic risk factor was known: the APOE gene, the product of which is an apolipoprotein E responsible for fat metabolism (Saunders et al., 1993). Interestingly, some other genetic risk factors are found to be gene expressed not in neurons but in other neural system cells such as microglia. Like for example this genomic research showed the association of mutations in alleles of these genes TREM2, ABI3, and PLCG2 (Consortium et al., 2017). All these genes even though being expressed in all cell types of the cortex show much higher expression levels in microglial cells. Gene products of them are building blocks of very complex pathways which if generally speaking all serve the immune function of microglia. This suggests the importance which these cells and, in general, immune functions play in the whole neurodegeneration process.

Other studies show also others possible risk-factor loci (Kamboh et al., 2012). And it is clear that further studies will show many more of them. Taking together, it is obvious that genetic underprinting in the sporadic form of AD is not homogenic. The genetic diversity of Alzheimer patients correlates well with already mentioned diversity of amyloid structure and clinical symptoms of disease progression.

Although there are far more genetically deterministic neurodegenerative disorders as well, such as Huntington's disease (Hoogeveen et al., 1993), most of the neurodegeneration diseases are the same heterogeneous in their genetics as is Alzheimer's. To conclude, although there are many correlations between genetics and neurodegeneration, it can not be called a genetic disorder since its symptoms are the result of much more complex interactions between many genetic factors and environment.

2.2 Unifying hypotheses of neurodegeneration

It is undisputed that there is a considerable correlation between amyloid or amyloid beta specifically, and Alzheimer's disease with all its clinical symptoms. Genetic research also points out the enormous importance of amyloid beta as the main constituent of later amyloid plaques, as has been discussed earlier. Thus,

for some authors, mutations that have an influence on Aβ length and solubility are the first step of neurodegeneration. Based on this and other data, Hardy J. and Higgins G., for example, in 1992 paper proposed the amyloid cascade hypothesis (Hardy and Higgins, 1992). According to them, the APP is the factor that initiates the neurodegeneration cascade. As some mutations influence the proteolysis of APP, thus, cause neurons to secrete more of the pathogenic form of $A\beta$ as they influence their surroundings causing damage to proximal neurons. In response to this damage, neurons alternate the calcium influx into the cell. As calcium is known to be the main second messenger of the cell and participates in many cellular regulation pathways, it could be reasonable that it influences the phosphorylation of tau protein as well, so generating intracellular tangles. When this happens, it leads also to cell death. In the same paper, however, the authors notice that their evidence is built on genetic studies of a familial form of AD . Genetic research of the sporadic form, as was mentioned, leads to the conclusion of other mechanisms possibly involved. 30 years later from this paper publication, even more possible mechanisms are opened, if based on the genetic research of SAD.

Other authors mention that $A\beta$ secretion may not be exactly an initiation factor but rather can be a normal physiological occurrence, based on evidence that amyloid deposits do not always correlate with cognitive decline (Edwards, 2019; Iacono et al., 2008).

There are also a series of different works focusing on other players of neurodegeneration. Microglial involvement could also shed light on what is going on.

The subject of debate is also the way how neurodegeneration is progressing, or how it propagates from its point of origin to almost all regions inside its brain area. Since almost the first research in this area, there is a question of similarity between neurodegeneration and prion-type diseases.

All the risen question are applicable not only for described forms of AD, but for any other neurodegenerative disorder. As, in general, most of them starts with genetics which is shown to not have any clear and easy causality but rather a complex network of interaction. All of them, show some kind of proteinous aggregations, rather from $A\beta$, synuclein or tau. And lastly, all seem to potentially spread though prion-like mechanism and disrupt neural networks.

3. Application of connectomics in studies of neurodegeneration

3.1 Biomarkers of neurodegeneration

As was mentioned in a previous chapter, neurodegeneration is culminated in thinning of a cerebral cortex due to cell loss; cell loss can not occur without connectivity reduction as soon as dying neurons' synapses also degenerate. Certain neurons are playing the linking or central processing role in networks and their death brings a potential risk of whole network disruption. Thus, it is said that connectivity disruptions are linked to upstream and downstream neurodegenerative processes. (processes laying upstream are molecular mechanisms that underlay neurodegeneration when observed clinical symptoms are said to lay downstream.) Connectivity disruptions are observed among many studied networks, both functional and structural, and they are seen in almost all known neurodegeneration diseases.

In Alzheimer's disease spectrum, the most prominent changes occur among functional networks: mainly, the activity of DMN is reduced in affected individuals (Jones et al., 2017). The network also shows significant overlap with areas primarily affected by tau and amyloid pathology or, in other words, with areas rich in tau and amyloid deposits (Buckner et al., 2005). This suggests a link to upstream events, though, the link is of uncertain causality; the most attractive hypothesis suggests that protein pathology affects networks, however, it could be explained and another way around, so that network activity could lead to proteinopathy. The localization of tau deposits and subsequent changes in the connectivity of the medial temporal lobe could explain the disease symptoms since this area is active in memory retrieval; this area also corresponds to the core of DMN that is also highly connected to other networks; the disruption of it leads to the loss of the small worldness property of the brain, in other words, the brain is less integrated and the mean length of pathways increases (Stam et al., 2007). Changes of hippocampus - cortex connectivity, including hippocampus connections to composite regions of DMN, correspond to memory formation complications observed in Alzheimer's patients (Wang et al., 2006). Moreover, some of the studies above report an increase in changes in network connectivities as the disease progresses. Structural connectivity provides a physical basis for the observed reduction in DMN functioning; it shows too the reduction of white matter pathways in the limbic system and in cortical-cortical connections, including the cingulum and the fornix -the major pathways coming through the medial temporal lobe (Pievani et al., 2010).

Similarly, in frontotemporal dementia FTD, the most affected network reported is the salience network, this is also confirmed by the reduction in underlying structural tracts (Zhou et al., 2010). Likewise the findings of AD studies, this data could be explained in accordance with the upstream and downstream events, since there is also an overlap of affected networks with physical localization of damage. Interestingly, the effect of FTD on networks is exactly the opposite of the effect of Alzheimer's, same as the observed clinical picture is also of opposite

effects. In Parkinson's disease, the most prominent changes are observed in the structural pathways of the brainstem and in thalamocortical pathways, with the progression of the disease seems to spread changes to structural connectivity of other areas as well, and in more severe cases the damage is observed in many areas including olfactory tracts, major interhemispheric, limbic, and extra motor association tracts (Agosta et al., 2013). Depending on the degree of connectivity disruption Parkinson's patients also demonstrate cognitive decline (Hattori et al., 2012).

It is worth mentioning that during normal healthy aging brain networks, both structural and functional, also do not tend to stay the same. And the observed change is similar to this in AD: the DMN shows lower activity; and similar to this in other neurodegeneration diseases: lower salience and sensorimotor networks (Damoiseaux, 2017). Generally, the older brain shows much higher integrity in different networks, and lower integrity inside networks.

The described network changes themselves can not, unfortunately, reveal us a clear picture of the disease onset and progression, since there are also studies that show, for example, that people with FAD given by mutations in APOE4, exhibit modified network's activity far before any disease manifestation (Quevenco et al., 2020). Or, similarly, some differences in young default brain activities accounted for future risk of AD development (Buckner et al., 2005). This would suggest that the observed connectivity abnormalities are not the results of ongoing, although early, neurodegeneration but are risk factors and results from the specific genetic composition.

Nonetheless, the described alternations can provide alternative biomarkers for disease recognition at early stages. Since symptoms appear first only after even decades of neurodegenerative processes, good biomarkers could be very valuable tools of diagnosis. They could provide information not only about possible risks but also localize them. With the additional help of further biomarkers, like CSF markers (typically $A\beta$ and tau), it can help to determine the stage and subtype of the disease (Vemuri et al., 2009). An interesting example comes from the study of connectivity change in patients with Parkinson's disease. The study compared drug-naive PD patients with those who were treated with levodopa, a popular medication against PD symptoms, and used knowledge of the rate of atrophy observed from connectivity changes. This provides the possibility to use connectivity alternations to test various drug efficiency against different disorders as an alternative to cognitive and behavioral tests which could be much slower and not reliable markers (Seeley et al., 2009; Esposito et al., 2013).

3.2 Transneural spread hypothesis

It was discussed earlier in the chapter that amyloid load localization overlaps with DMN activity, this is shown in many studies. It was also mentioned that some carriers of Alzheimer-associated mutations exhibit changes in brain network activity even when not affected by the disease. For example, APOE $\epsilon 4$ carriers show increased functional connectivity of the medial temporal lobe with the region of DMN - posterior cingulate (PCC), and other peri-limbic regions (Dennis et al., 2010). Both regions serve for memory retrieval and are reported to be damaged by tau and Aβ deposition in Alzheimer's. These data suggest the existence of

a backward correlation between networks and proteinopathy. It proposes that network activity can regulate and guide future proteinopathy if such is at risk, and raises the question of how exactly can network activity in a healthy brain state predict future neurodegeneration process and severity.

Prion diseases are diseases stimulated by the appearance of prions in the CNS, such diseases are, for example, scrapie in sheep, mad cow disease, or Creutzfeldt–Jakob disease (CJD) in humans. In a certain sense, prion diseases are also the same as neurodegeneration proteinopathy, since it starts with protein misfolding. Prion diseases, however, are different from neurodegeneration because they are considered infectious. Certain misfolded proteins can affect other these proteins in a native conformation and turn them to conformational change (Prusiner, 1982; Pan et al., 1993). These proteins spread involving more proteins in a misfolding state and creating aggregates much similar to that of neurodegeneration. As this happens, different brain areas get affected and collapse their functionality at some point. This disease is considered fatal, and, similarly to neurodegeneration in humans, mainly appears in older age.

Neurodegeneration in the brain always starts in a certain area and proceeds in development in a predictable manner. The mechanism of neurodegeneration spread still remains unclear, but increasing evidence is made of so-called transneuronal spread. This hypothesis states that the misfolded protein aggregates in neurodegenerative diseases spread along with existing neural networks by mechanisms similar to that of prions, so that each next affected protein is misfolded with the aid of previous, and via cell-to-cell transmission, they travel through neurons. This was partially proved by experimental science and showed amyloid prion-like action on in vitro models (Baker et al., 1993), or transneuronal tau transmission in vivo (Liu et al., 2012). Additional proof to this hypothesis comes from connectomics. First of all, the motivation for this hypothesis comes from already described and widely observed data of overlapping protein deposits and existing neural networks and their activity. Secondly, a series of studies confirmed this hypothesis with resting-state fMRI data. Central nodes with minimal path length to all other nodes, and specifically closer to disease epicenters, in networks, show greater vulnerability to disease, since their location makes them closer to the disease onset place and, thus, they are first to be affected in the transneuronal spread hypothesis. The same is true for other network nodes, that are usually affected later in disease progression. Those that are closer to epicenters are more at risk (Zhou et al., 2012). Importantly, models built on the basis of transneuronal transmission are able to predict future layout of brain atrophy and damage in real life patients (Raj et al., 2012).

There are also alternative hypotheses of neurodegeneration spread, such as the hypothesis of nodal stress, trophic failure hypothesis, or shared vulnerability hypothesis (Zhou et al., 2012). All of them make sense in the light of the connectomics perspective and require further investigation using connectomics methods. Therefore, connectomics provides not only "supportive" research but can be helpful to answer very fundamental questions about the nature of brain disease.

3.3 Networkopathy

Neurodegenerative diseases were a long time conceptualized on the subsystem level. The pathology was studied separately from the place where it occurs. The diseases are used to be called proteinopathies since for a long time it was known that they start with misfolding and aggregation of certain proteins. They were used for medical diagnosis and actively studied. They could not, however, fully explain the observed clinical signs, same as the subsystem approach, cannot fully explain the whole phenomenon. Symptoms could vary person-to-person, although the underlying protein pathology would be the same. The systems approach and the network perspective are able to describe the observed symptoms and to explain how the small events of protein misfolding translate into whole-brain disease. And the connectomical data are aimed to help us to look at the brain systematically. They are vital for our understanding of the disease: only they can show how a certain phenotype is created, only they can help to trace the spread of pathology. The fact that a higher education level decreases the risk of neurodegeneration suggests (Roe et al., 2007) that network redundancy protects the brain from this process; and motivates for looking for possible treatments with network approaches too.

After all, the findings described in this chapter have several implications not only for the study of neurodegeneration. They show us a new approach to all brain diseases including such as schizophrenia and depression.

Conclusion

In this thesis I have described what is connectomics. I went through its theoretical basis including its methods and main tools of analysis. I showed what progresses neuroscientists achieved in this field with the example of *C. elegans* and human connectome. I went through the main concepts of neurodegeneration, even though a lot seems to be still unknown on this topic. Finally, I have shown how using connectomics we can answer the fundamental questions about neuropathology; I demonstrated the system-based proof of the transneural spread hypothesis. Moreover, I discussed the possible usage of connectomics in clinical practice.

All of this should show the potential of a connectomical approach, since it can be applied for all known neuropathologies. It can be also used in fundamental studies of cognition as it can reveal how the brain operates. An interesting topic for future research would be the question of brain network evolution. I would speculate that a lot of evolutionary constraints are invisible at the molecular and anatomical levels and are given purely by network topology rules, so that it would make it nearly impossible to understand brain evolution without diving deep into connectomics.

On the contrary, it is clear, and many researchers point it out, that connectomics has extremely ambitious goals. And they cannot be achieved fully in the current technological state. Moreover, it is, for the moment, disintegrated; many research groups work on different levels of it, with different methods. Public integrated database exists but still appears to be more a collection of separate research results.

But the Human Genome Project should teach us that if scientific community cooperates, we, as humanity, are able to achieve the most ambitious goals and connectomics should not be exception.

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Internet resources

[1] Eyewire game: https://eyewire.org/explore
[2] Online database of *C.elegans* connectome: https://wormatlas.org
[3] Human connectome online database:
http://www.humanconnectomeproject.org
[4] Graphic content taken from Protein Data Bank structural information on proteins: http://pdb101.rcsb.org/motm/189 Uploaded on 27/04/2021
[5] Graphic content taken from Harvard University Blog:
https://sitn.hms.harvard.edu/flash/2016/can-computers-use-brain-scans-to-diagnose-psychiatric-disorders Uploaded on 27/04/2021
[6] Graphic content taken from Wikipedia page on diffusion MRI:
https://en.wikipedia.org/wiki/Diffusion_MRI Uploaded on 27/04/2021
[7] Graphic content taken from Wikipedia page on default mode network: https://en.wikipedia.org/wiki/Default_mode_network Uploaded on 27/04/2021